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Comparative morphology of the genus *Chilasa* (Lepidoptera, Papilionidae)

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Abstract A total of 12 species of the Oriental mimetic swallowtail genus, *Chilasa*, was studied. Comparative morphological analysis of the male and female genitalia supported monophyly of the genus *Chilasa*. It also became clear that the genus is made up of 5 monophyletic species-groups and one enigmatic group. This result was almost consistent with the phylogenetic hypothesis based on molecular analysis of genomic nucleotide sequences.

Key words Phylogeny, morphology, species group, Batesian mimicry, molecular phylogeny.

Introduction

Species of the Oriental swallowtail genus, *Chilasa* (*sensu* Hancock, 1983), are distributed from South China to Papua New Guinea. All species that have been included within *Chilasa* have a remarkable resemblance to some dominant butterflies in the Danainae or to a Uraniid moth. Although one ecological character, distinct mimicry to other species, is shared among the constituents, it is too ambiguous to define the genus systematically. Hence, since the establishment of the genus, all attempts to devise clear criteria defining its Batesian mimicry morphologically have fallen flat.

Since Batesian mimicry in butterflies has been recognized as consisting of mimetic patterns of wing color, wing shape and flight pattern, most studies on *Chilasa* have focused on these traits. However, it is possible that strong selection towards similarity to model species caused an evolutionary tendency of these characters under the influence of non-phylogenetic factors, because it is not necessary that the phylogenetic relationships of model species reflect those of mimetic species. Actually, most early studies that focused on wing characters failed to establish a relevant definition of the genus. Therefore in this study, morphology of the genitalia which are supposed to be more stable than wing characters was brought to bear.

Historical review

Moore (1881) established the genus *Chilasa* as a monobasic genus represented by "*Chilasa dissimilis*" that corresponded to the present *Chilasa clytia dissimilis*. Moore (1903–1905) recognized 9 species in *Chilasa*, which, on present criteria, form variations of *Chilasa clytia*. In addition to that, Moore (1903–1905) stated about the genera *Euploeopsis*, *Cadugoides*, and *Menamopsis* (or *Isamiopsis*), respectively represented by the present species, *Chilasa paradoxa*, *C. epycides* and *C. slateri*. The reason of this fragmentation was based on the extraordinarily variable color patterns within one species (*e. g. Chilasa clytia*, *C. paradoxa*).

Except for Talbot (1939) who interpreted *Chilasa* based on wing venation pattern, the rest of the authors treating *Chilasa* as an independent genus claim the distinctiveness of their early stages (Ford, 1944; Shirôzu, 1960; D'Abrera, 1971, 1981, 1982; Pinratana, 1977; Tsukada and Nishiyama, 1980; Hancock, 1983; Igarashi, 1966, 1976, 1984; Igarashi and

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Fukuda, 1997; Otsuka, 1988; Yamamoto *et al.*, 2000). The latest and comprehensive study of *Chilasa* was by Hancock (1983) who recognized 2 subgenera consisting of 5 species groups within the genus, among which the subgenus *Agehana*, including one species group, the *elwesi* group, was not regarded as a constituent of the genus in Igarashi (1984). Although 4 species groups (the *agestor*, the *clytia*, the *veiovis* and the *laglaizei* groups) have been *recognized* stably within *Chilasa*, interpretations of the *anactus* group (Tsukada and Nishiyama, 1980), the *anchisiades* group (Igarashi, 1984) and the *elwesi* group (Hancock, 1983) have been variable among authors. Additionally, if the *anchisiades* group is included in the genus, the generic name "*Chilasa*" must be turned into "*Primides*" (Yoshimoto, 2000).

Among successive studies dealing with classification of the Papilionidae, there has been a tendency not to recognize *Chilasa* as a genus (*e. g.* Collins and Morris, 1985; Bridges, 1988; Persons, 1998). In a number of studies *Chilasa* was not recognized as a coherent genus because it was not thought to have sufficient distinct characters to allow it to be treated as an independent genus (Jordan, 1908–1909; Bryk, 1930; Munroe, 1960). As the criteria that have been employed to distinguish *Chilasa* from other Papilionidae genera, especially *Papilio*, have not provided a high enough degree of discrimination, *Chilasa* tends to be recognized as one of the subgenera of the genus *Papilio*.

Materials and methods

The materials used in the present study principally consisted of dried specimens stored in the Biosystematics Laboratory, Kyushu University (BLKU), the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka (ELKU) and Osaka Natural History Museum (ONHM).

Morphological analysis

Abdomens were separated from the rest of the adult body and soaked in a 40% aqueous solution of KOH at 50°C for 3 h. After the KOH treatment, the preparation was dissected under SZ60 (Olympus).

Relationship among taxa was evaluated mainly on the basis of comparative analysis of exoskeletal features of the adult genitalia. A total of 12 species of swallowtail butterflies that have been recognized as constituents of the genus *Chilasa*, were examined (*agestor*, *epycides*, *slateri*, *clytia*, *paradoxa*, *osmana*, *veiovis*, *laglaizei*, *toboroi*, *elwesi*, *maraho*, *anchisiades*). Additionally, *Papilio machaon*, which is the type species of the genus *Papilio*, was also analyzed for generic level comparison.

The terminology of morphological characters was basically from Miller (1987) except where some ambiguity arose, where, on a case by case basis, we employed the terminology proposed by Saigusa *et al.* (1977) and Smith and Vane-Wright (2001).

Results

Comparative morphology of male genitalia

Most species that had been included in the genus *Chilasa*, except one species (*slateri*) had a round harpe at the inner side of each valve (Figs 1–2, 4–13). In addition, among the examined species except *anactus* and *anchisiades*, but including *slateri*, a serrate edge of the harpe was shared (Figs 1–9, 12–13). In the species examined, only *slateri* had an arrowhead-shaped harpe that resembled the shape of the harpe of *Papilio* species (Figs 3, 14). Apart from this, the regularly indented harpe of *anactus* and *anchisiades* was simpler than

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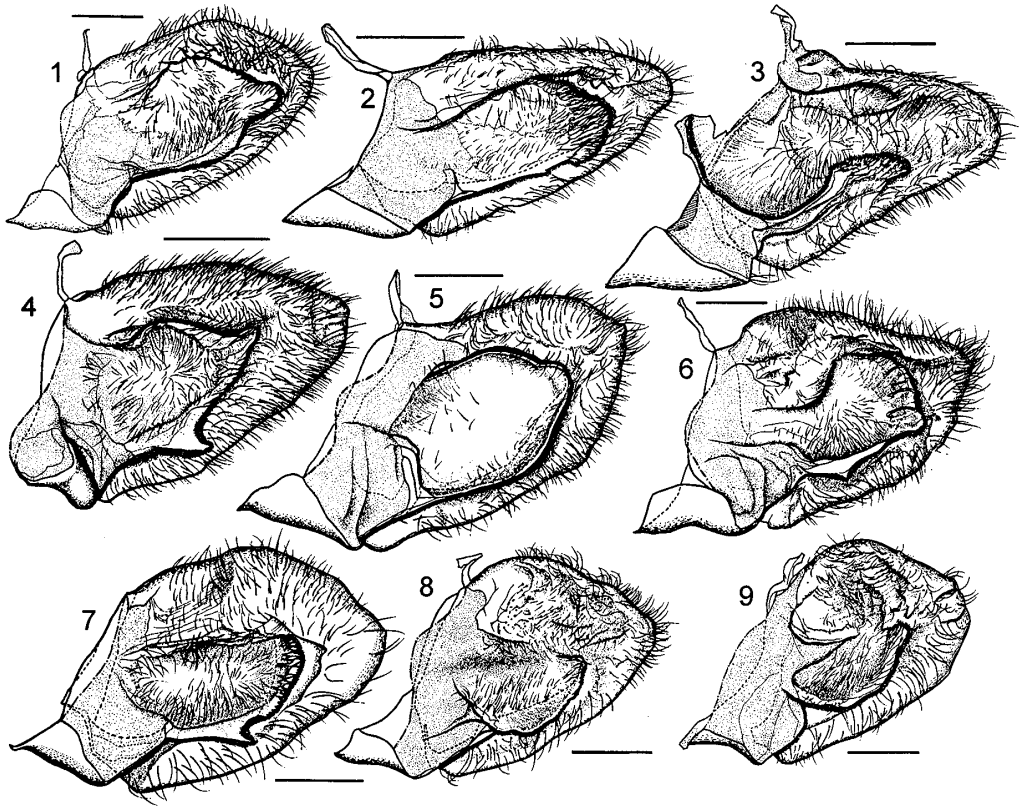

Figs 1–9. Valva of species of *Chilasa*. 1. *C. agestor*. 2. *C. epycides*. 3. *C. slateri*. 4. *C. clytia*. 5. *C. paradoxa*. 6. *C. veiovis*. 7. *C. osmana*. 8. *C. laglaizei*. 9. *C. toboroi*. Scale lines: 1.0 mm.

that of the other species, which have partly rugal, irregular serration (Figs 10, 11). The nesting distribution of specialized structures of the harpe made it difficult to decide taxonomic positions of controversial groups (the *elwesi*-, the *anactus*-, and the *anchisiades* groups), which indicated that male genital traits could be no more than one source of suggestions on defining the generic species range of *Chilasa*.

As for species-groups, no common criteria that could define each group were found. However, some particular structures were found in some groups. For instance, species of the *veiovis* group have a derived ledgy structure on the inner side of the harpe (Figs 6, 7).

Comparative morphology of the female genitalia

Several unique genital structures appeared to be shared among females of the *Chilasa* species (Fig 15–22). First, complex sclerotized projections called 'lobes' (Saigusa *et al.*, 1977) are developed around the ostium bursae. Second, the ventro-medial projection, 'central lobe' and the ventro-lateral projections, 'lateral lobes' are recognizable, and they are fused to one another in various ways as well. In species of *Papilio*, *Meandrusa* and *Graphium*, the central lobe is not developed, while lateral lobes strongly develop (Fig. 27). Third, the surface of sclerotized invagination into which the male pseuduncus is inserted on copulation is very rugal in appearance compared with other papilionids.

Within the problematic groups, the *elwesi*, the *anactus* and the *anchisiades* groups, the former two lacked a central lobe and have only lateral lobes and sclerotized invagination with

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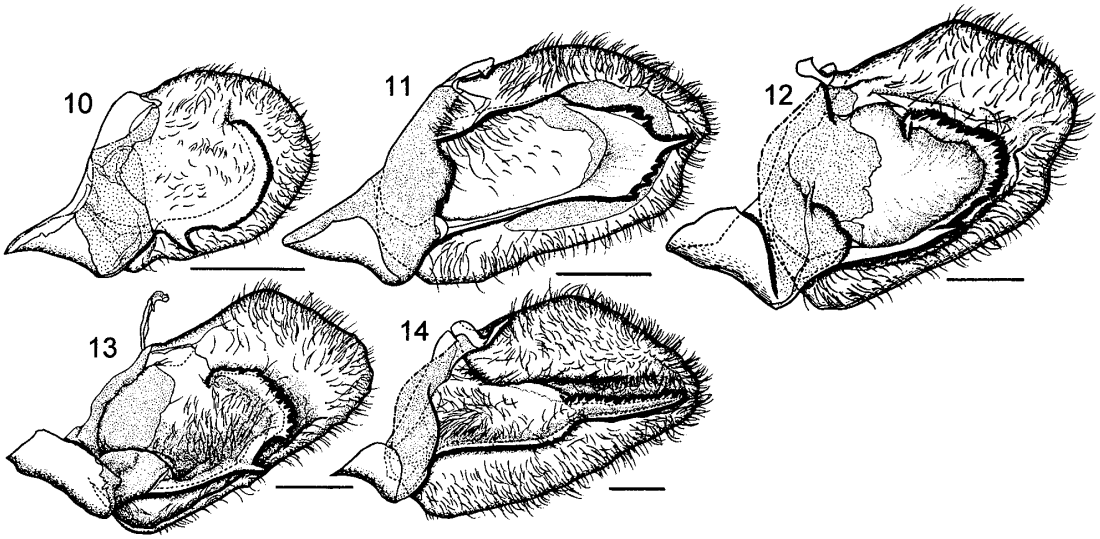

Figs 10–14. Valva of that were taxonomically related to *Chilasa*. 10. *Eleppone anactus*. 11. *Heraclides anchisiades*. 12. *Agehana elwesi*. 13. *A. maraho*. 14. *Papilio machaon*. Scale lines: 1.0 mm.

few rugae, and the last group was equipped with an advanced central lobe which was free from conglutination with the lateral lobes and with an intricate sclerotized structure on the dorsal side (Figs 23–26). Their morphological distinctiveness made it clear that they should be excluded from the genus *Chilasa*. Hence, on account of female genital characters, the genus could be defined to include Hancock's (1983) subgenus *Chilasa*.

Hancock (1983) divided the subgenus *Chilasa* into 4 species groups: morphological analysis of the female lobes did not support the *agestor* group and the *clytia* group, while the *veiovis* group and the *laglaizei* group were highly supported. In detail, the female of *C. agestor* had a developed central lobe and lateral lobes fused each other to form a calyx-shaped projection dorsally covered with the broad, isolated posterior labia of the vestibulum, while the other members of the group (*C. epycides*, *C. slateri*) had tiny central lobes and the posterior labia of the vestibulum were conglutinated with the lateral lobes. As for the *clytia* group constituents, the female of *C. paradoxa* had an extraordinarily elongated bifid central lobe that did not exist either in *C. clytia* or in other species of Papilionidae.

Discussion

Morphology of male and female genitalia

Hancock (1983) recognized 2 subgenera and 5 species groups within the genus *Chilasa* based on male genital morphology, larval morphology, and food plants. However, as Miller (1987) noted, the generic definition given by Hancock suffered from a bias towards modified characters that were supposed to be strongly influenced by mimicry. Furthermore, Hancock's interpretation had its basis on Munroe (1960) except for inclusion of the subgenus *Agehana* into *Chilasa*, which obviously reflects Igarashi's (1976) opinion. Such a chimeric classification system included logical contradictions among the criteria in nature.

The present morphological study has shown that morphological traits in the male and female genitalia of species in the subgenus *Agehana* including the *elwesi* group (Hancock, 1983) are clearly distinguishable from species within the subgenus *Chilasa* on the basis of the following points. First, in the male genitalia, the margin of the harpe is indented but not

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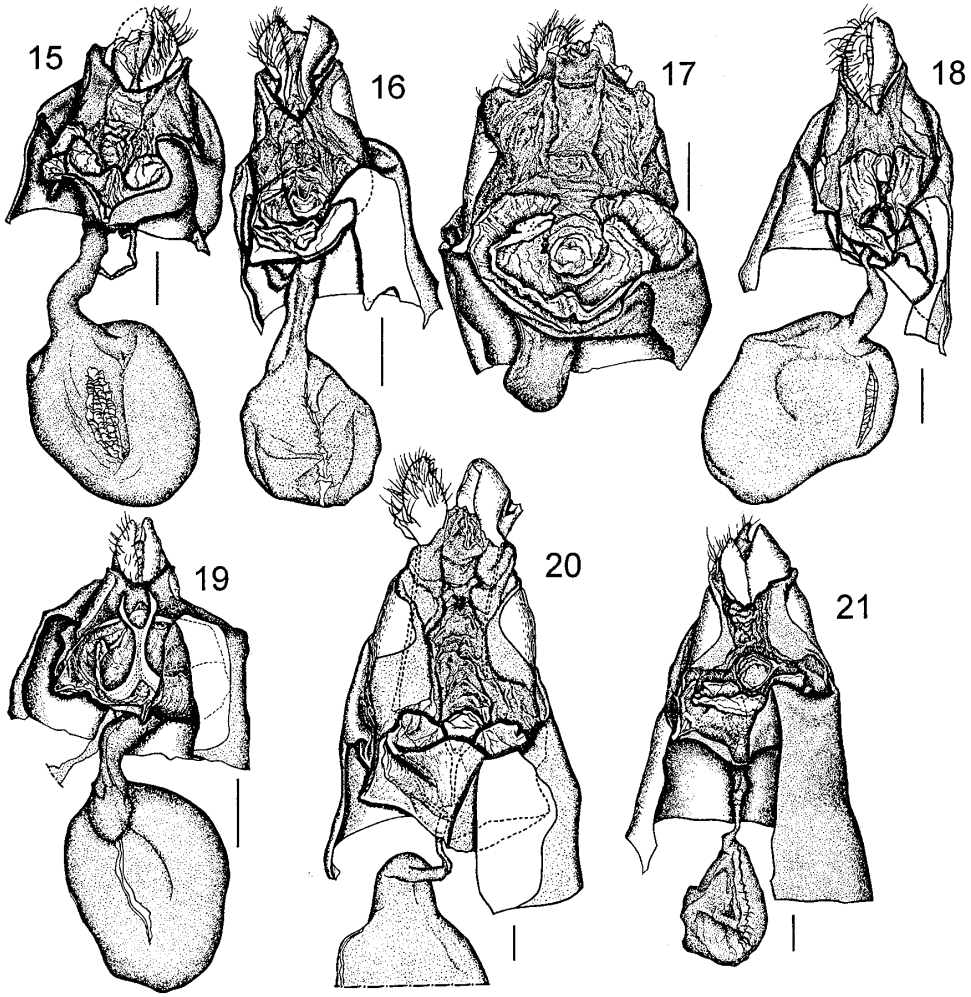

Figs 15–21. Female genitalia of species of *Chilasa*. 15. *C. agestor*. 16. *C. epycides*. 17. *C. slateri*. 18. *C. clytia*. 19. *C. paradoxa*. 20. *C. veiovis*. 21. *C. osmana*. Scale lines: 1.0 mm.

rugal (Figs 12, 13). Second, in the female genitalia, the inner areas of the lateral lobes are poorly developed especially around the central lobe and never form a cup-like projection. Third, the sclerotized invaginations of the females were not clear (Figs 25, 26). Hence the authors conclude that these male and female genital features are distinctive enough to exclude Hancock's (1983) subgenus *Agehana* from the genus *Chilasa*, even when taking account of some resemblances in the early stages (*cf.* Igarashi, 1976).

The second controversial group, the *anchisiades* group (Igarashi, 1984), is obviously different from species in *Chilasa* because of the complicated structure of the female genital lobes (Fig. 24).

The third uncertain group, the *anactus* group (Tsukada and Nishiyama, 1980), also differs from the species of *Chilasa* for the same reason as the *elwesi* group addressed above (Figs 10, 23).

The female genitalia of *Eleppone anactus* and species of *Agehana* were found to share the following character with that of species belonging to the genus *Papilio*; in their female genitalia, except for the inner area of the lateral lobe surrounding the ostium, only the outer area of the lateral lobe is sclerotized (Figs 23, 25 and 26).

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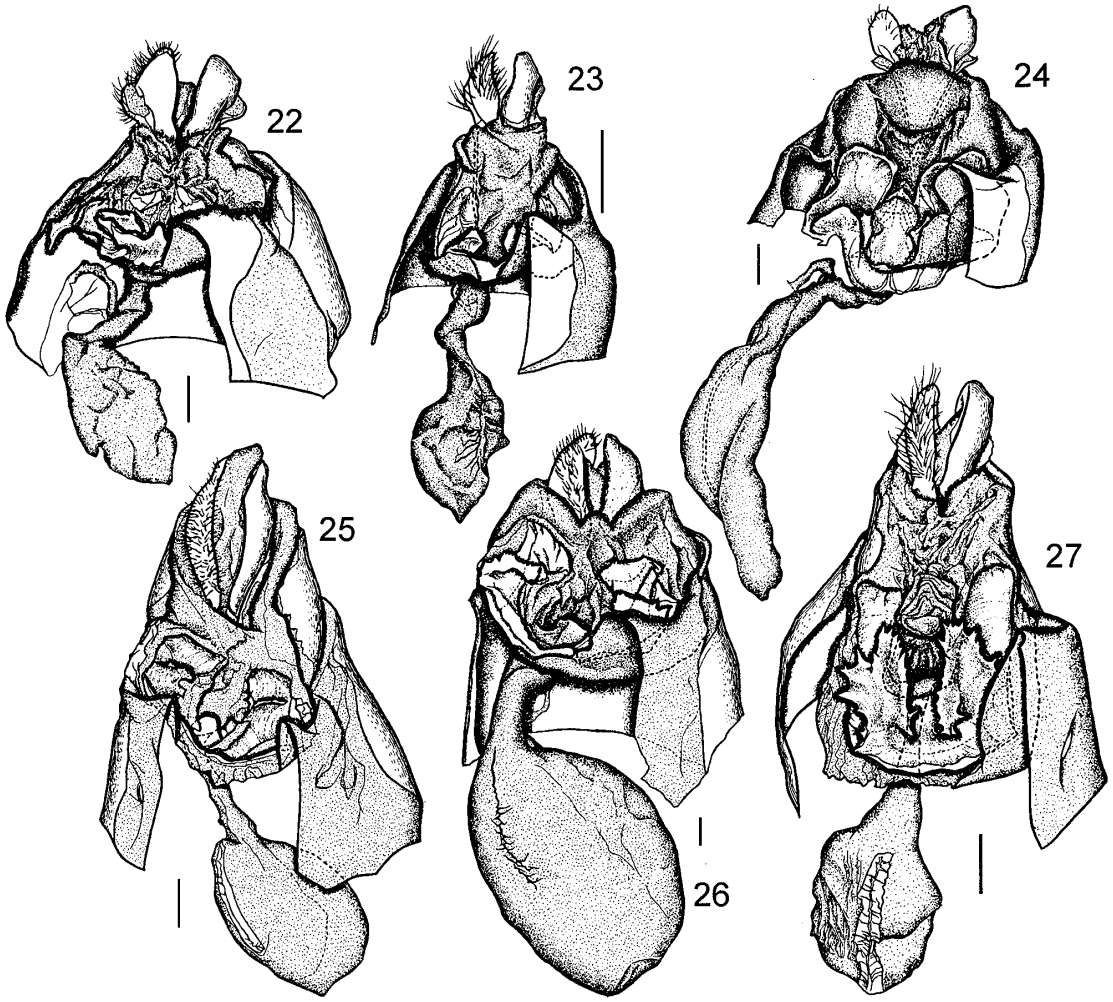

Figs 22–27. Female genitalia of species of *Chilasa* and the taxonomically related species. 22. *Chilasa laglaizei*. 23. *Eleppone anactus*. 24. *Heraclides anchisiades*. 25. *Agehana elwesi*. 26. *A. maraho*. 27. *Papilio machaon*. Scale lines: 1.0 mm.

Thus, the genital comparison addressed above suggests the hypothesis that *Chilasa* might consist of Hancock's (1983) subgenus *Chilasa*, including 4 species groups (the *agestor*, the *clytia*, the *veiovis* and the *laglaizei* groups).

Accordingly, interspecific comparison of the species within *Chilasa* revealed the following facts.

First, in terms of the genital morphology, the *veiovis* group and the *laglaizei* group were strongly supported. The *veiovis* group possesses notable derivative characters both in the male and female genitalia (Figs 6, 7, 20 and 21). Meanwhile, as for the *laglaizei* group, though female synapomorphic characters could not be ascertained because the female of *C. toboroi* was not examined in the present study, the male genital morphology of the two constituent species was strikingly homogenous (Figs 8, 9 and 22).

Second, the *agestor* group was poorly supported because the three constituent species differed from one another especially in genital characters (Figs 1–3, 15–17). In addition to that, the morphological distinctiveness of the male genitalia of *Chilasa slateri* suggests that *C. slateri* might possibly be more distantly related to the rest of the species within *Chilasa*

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than it had been thought (Figs 3, 17).

In addition, and in contrast, there exist some resemblances in female genitalia between *C. epycides* and *C. slateri*, but the female genitalia of *C. agestor* have little in common with the former two species of the *agestor* group, and rather have something in common with the female genitalia of species in the *veiovis* group in that a notable calyx-shaped projection and an obvious central lobe are developed.

Third, *C. paradoxa*, which had been supposed to form the *clytia* group with *C. clytia*, was found to possess extraordinary unique morphological traits both in the male and female genitalia (Figs 5, 19). These genital characters of *C. paradoxa* suggest two possibilities. 1) *C. paradoxa* may actually be a more derivative species than the other species of *Chilasa*, on the basis of a difference between the evolutionary rate of genitalia and that of wing appearance. 2) These conspicuous genital characters of *C. paradoxa* may have their origins in secondary morphological changes without the influence of phylogenetic factors.

In conclusion, the following intrageneric classification system could be proposed: the *agestor* group (*C. agestor*); the *epycides* group (*C. epycides*); the *slateri* group (*C. slateri*); the *clytia* group (*C. clytia*); the *paradoxa* group (*C. paradoxa*); the *veiovis* group (*C. veiovis*, *C. osmana*); the *laglaizei* group (*C. laglaizei*, *C. toboroi*). As shown above, from a morphological point of view, the previous *agestor* and *clytia* groups are divided into three and two monobasic species groups, respectively.

To define the genus *Chilasa* on the basis of the generic structure outlined above, the criteria will naturally depend on what extent characters are held in common among these groups within the genus. Then the most problematic morphological genital definition of *Chilasa* is as follows. 1) Male genitalia are equipped with serrate and rugal margin of harpe. 2) Female genitalia exhibit a wide variety of conglutination between the central lobe and the lateral lobe which forms the calyx-shaped sclerotized projection. If these genital characters are regarded as synapomorphies, *Chilasa* can be a phylogenetically guaranteed monophyletic genus.

Taxonomic conclusion

Revising the classification of the genus *Chilasa* using genital characters, the monophyly of the genus *Chilasa* was almost supported, though it was also found that the subgenus *Agehana* that had been included in *Chilasa* by Hancock (1983) should more appropriately form a separate genus, *Agehana*. In addition, it is also suggested that other controversial species groups, the *anchisiades* group (Igarashi, 1984), and the *anactus* group (Tsukada and Nishiyama, 1980), should be excluded from the genus *Chilasa*, which would overcome the generic name problem that was mentioned in Yoshimoto (2000).

In the subgeneric classification, both the *laglaizei* and the *veiovis* groups were highly supported morphologically, while the *agestor* group was poorly supported. Hence, in comparison with the former two groups, it could be concluded that the *agestor* group could not be recognized as one species group and that it should be divided into three monobasic groups; the *epycides*, the *slateri* and the *agestor* groups.

As for the *clytia* group, the hypothesis derived from morphological analysis and that from molecular data contradict each other (Morinaka, *unpubl.*). It was found to be totally heterogeneous in morphological terms, while molecular data strongly supported the monophyly. Thus more intensive and multipronged investigation is required in order to solve the incongruence between morphology and molecular phylogeny.

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References

Bridges, C. A., 1988. *Catalogue of Papilionidae and Pieridae (Lepidoptera, Rhopalocera)*. 721 pp. Urbana, III, Carles A. Bridges.

Bryk, F., 1930. Papilionidae III (*Papilio*). *Lepid. Cat.* **39**: 217–218, 591–596. W. Junk, Berlin.

Collins, N. M. and M. G. Morris, 1985. *Threatened Swallowtail Butterflies of the World.* IUCN, Gland and Cambridge.

D'Abrera, B. L., 1971. *Butterflies of the Australian Region.* Lansdowne, Melbourne.

————, 1981. *Butterflies of the Neotropical Region.* Part 1. Papilionidae & Pieridae. Lansdowne, Melbourne.

————, 1982. *Butterflies of the Oriental region.* Part 1. Papilionidae, Pieridae, Dannaidae. Ferry Creek, Australia.

Ford, E. B., 1944. Studies in the chemistry of pigments in the Lepidoptera, with reference to their bearing on systematics. 4. The classification of the Papilionidae. *Trans. R. ent. Soc. Lond.* **94**: 201–223.

Hancock, D. L., 1983. Classification of the Papilionidae: a phylogenetic approach. *Smithersia* **2**: 1–48.

Igarashi, S., 1966. Swallowtails of the world (1): Notes on the classification of the Papilionidae on the basis of the morphology of their immature stages. *Insects Nature* **4**: 4–8 (in Japanese).

————, 1976. What characterizes the genus *Chilasa*? It is represented in South America. *Yadoriga* (87/88): 3–16 (in Japanese).

————, 1984. The classification of the Papilionidae mainly based on the morphology of their immature stages. *Tyô Ga* **34**: 41–96.

Igarashi, S. and H. Fukuda, 1997. *The Life Histories of Asian Butterflies.* **1**. 549 pp. Tokai University Press, Tokyo.

Jordan, K., 1908–1909. Papilionidae. *In* Seitz, A. (Ed.), *Gross-Schmett. Erde* **9**: 11–118, pls 1–50. Stuttgart.

Miller, J. S., 1987. Phylogenetic studies in the Papilioninae (Lepidoptera: Papilionidae). *Bull. Am. Mus. nat. Hist.* **186**: 365–512.

Moore, F., 1881. *Lepidoptera of Ceylon.* Lovell Reeve and Co., London.

————, 1903–1905. *Lepidoptera Indica. Rhopalocera. Family Papilionidae. Sub-Family Papilioninae* (continued). **6**: 73–108, pls 496–514. Lovell Reeve & Co., Limited, London.

Munroe, E., 1960. The classification of the Papilioniadae. *Can. Ent.* (Suppl.) **17**: 1–51.

Otsuka, K., 1988. *Butterflies of Borneo.* **1**. 62 pp. Tokyo.

Persons, M., 1998. *The Butterflies of Papua New Guinea. Their Systematics and Biology.* xvi, 736 pp., XXVI, 136 pls. Academic Press, London.

Pinratana, B. A., 1977. Papilionidae and Danaidae. *Butterflies in Thailand* **1**: 21–28. Bros. St. Gabriel, Thailand.

Saigusa, T., Nakanishi, H., Shima, H. and O. Yata, 1977. Phylogeny and biogeography of the subgenus *Graphium* Scopoli (Lepidoptera: Papilionidae, Graphium). *Acta Rhopalocerologica* **1**: 2–32 (in Japanese).

Shirôzu, T., 1960. *Colored Illustrations of the Butterflies of Taiwan.* Hoikusha, Tokyo. (In Japanese).

Smith, C. R. and R. I. Vane-Wright, 2001. A review of the afrotropical species of the genus *Graphium* (Lepidoptera: Papilionidae). *Bull. nat. Hist. Mus. Lond.* (Ent.) **70**: 503–719.

Talbot, G., 1939. *The Fauna of British India, including Ceylon and Burma* (Butterflies) **1**. xxix, 600 pp., 3 pls. Taylor and Francis, London.

Tsukada, E. and Y. Nishiyama, 1980. Papilionidae. *In* Tsukada, E. (Ed.), *Butterflies of the South East Asian Islands* **1**: 287–296, 440, pls 102–107. Tokyo.

Yamamoto, T., Yata, O. and T. Itioka, 2000. Description of the early stages of *Chilasa paradoxa* (Zinken, 1831) from Northern Borneo (Lepidoptera: Papilionidae). *Ent. Sci.* **3**: 627–633.

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Yoshimoto, H., 2000. Laglaize's swallowtail (Papilionidae), a *Chilasa* or a *Primides* or a *Heraclides*? *Butterflies* (25): 40–47 (in Japanese).

摘　　要

マネシアゲハ属 (*Chilasa*) (チョウ目, アゲハチョウ科) の比較形態学 (馬田英典・矢田　脩)

熱帯東洋区を中心に分布し, ベーツ型擬態を行うことで知られているアゲハチョウ科の1属, マネシアゲハ属 *Chilasa* 12種についての分類学的再検討を行った. 雌雄交尾器による比較形態学的研究を行った結果, 本属の単系統性が支持され, 同時に, 本属は5つの単系統的な種群 (*agestor* 群, *epycides* 群, *slateri* 群, *veiovis* 群, *laglaizei* 群) とひとつの単系統性が明らかでない *clytia* 種群より構成されていることが明らかになった. これらの結果は, 森中 (未発表) による核遺伝子塩基配列情報から導出された分子系統仮説の結果とおおむね一致するものである.

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